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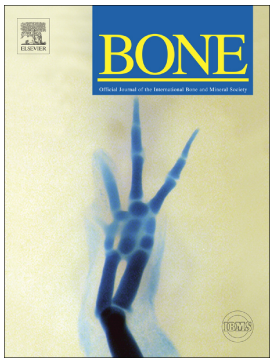

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In vitro osteoclastogenesis from Gaucher patients´cells correlates with bone mineral density but not with Chitotriosidase

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Abstract

Gaucher Disease (GD) is caused by mutations on the gene encoding for the lysosomal enzyme glucocerebrosidase. Type I GD (GD1) patients present anemia, hepatosplenomegaly and bone alterations. In spite of treatment, bone alterations in GD patients persist, including poor Bone Mineral Density (BMD). Mechanisms leading to bone damage are not completely understood, but previous reports suggest that osteoclasts are involved. Chitotriosidase (CHIT) is the most reliable biomarker used in the follow up of patients, although its correlation with bone status is unknown. The aim of this work was to study the pro-osteoclastogenic potential in patients and to evaluate its correlation with CHIT activity levels and clinical parameters. PBMCs from treated patients and healthy controls were cultured in the presence of M-CSF, and mature osteoclasts were counted. BMD, blood CHIT activity and serum levels of CTX, BAP, and cytokines were evaluated in patients. We found that blood CHIT activity and osteoclast differentiation were significantly increased in patients, but no correlation between them was observed. Interestingly, osteoclast numbers but not CHIT, presented a negative correlation with BMD expressed as Z-score. CTX, BAP and serum cytokines involved in bone remodeling were found altered in GD1 patients. These results show for the first time a correlation between osteoclast differentiation and BMD in GD1 patients, supporting the involvement of osteoclasts in the bone pathology of GD1. Our results also suggest that an altered immune response may play an important role in bone damage.

Key words: Gaucher Disease, bone pathology, osteoclasts, chitotriosidase, bone mineral density.

1. Introduction

Gaucher disease (GD) is an autosomal recessive disorder caused by deficient activity of the lysosomal enzyme glucocerebrosidase (GCase) due to pathogenic mutations in *GBA1* gene. This deficiency leads to accumulation of glucosylceramide mainly in macrophages, which convert into the so-called "Gaucher cells". The commonest phenotype is type I GD (GD1) which consists of visceral, hematological and skeletal alterations. About 90% of patients present bone affection characterized by Erlenmeyer flask deformity, reduced bone mineral density (BMD), bone infarcts, osteosclerosis, avascular necrosis, osteolytic lesions and pain. These alterations result in functionality and mobility disorders with reduction in quality of life (1,2). Bone disease results from a disruption of the balance between osteoblastic bone formation and osteoclastic bone resorption. However, the pathological mechanisms of bone alterations in GD are still poorly understood, and its knowledge would result in better treatment for patients. Pathophysiology is multifactorial, being fundamental the study of the relationship between bone marrow cells, Gaucher cells and bone cells. Evidence has demonstrated that the instauration of enzyme replacement therapy (ERT) substantially improves cytopenia, visceromegaly, growth (in children) and bone pain, reducing irreversible complications such as avascular necrosis, especially when initiated early (3–5). However, bone tissue is in some degree refractory to therapy (6,7).

Usual methods for the evaluation of bone alterations are radiology of femurs, thoracic and lumbar spine, BMD determination by dual-energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) of femurs and spine with the application of different available scores, such as Bone Marrow Burden score (BMB) in order to quantify bone marrow infiltration (8–10). Biochemical studies include markers of bone remodeling such as C-terminal telopeptide of type I collagen (CTX), a bone resorption marker, and bone alkaline phosphatase (BAP), a bone formation marker. However, different studies have shown highly variable results about these biomarkers in GD patients both, at baseline and during ERT (11).

Bone remodeling is closely regulated by the immune system (12). Several cytokines regulate bone dynamics, including the differentiation and survival of osteoclasts. Among them, TNFα is known as a potent inductor of osteoclastogenesis (13); while IL-10, TGFβ and IFNγ act as early inhibitors of osteoclast differentiation (14,15). On the other hand, IL-6 is a pleiotropic cytokine that enhances RANKL expression in osteoblast and stromal cells but it is negative regulator of osteoclasts´maturation (16). In this context, it is worth pointing out that glucocerebroside accumulation in GD can induce macrophage activation and secretion of cytokines such as IL-6, IL-1β and TNFα (17,18). This altered cytokine environment created by macrophages as well as

dysfunctions on other immune cells (19), would favor bone resorption and inhibit bone formation (20,21). Indeed, increased levels of osteoclast precursors in peripheral blood mononuclear cells (PBMC) from GD patients with higher tendency to differentiate into functional osteoclasts were recently revealed (22,23). In addition, osteopenia caused by reduced bone formation was demonstrated in a murine model of GD (24).

Chitotriosidase (CHIT) is the classical biomarker used in the follow up of the treatment of patients with GD. CHIT is an enzyme secreted by Gaucher cells, and it is elevated in untreated Gaucher patients. Plasma CHIT is thought to reflect the total body burden of Gaucher cells and it correlates with several clinical parameters of GD. Positive correlations between CHIT and organ volumes, and negative correlation between CHIT and platelet count have been described (25). Nevertheless, the relationship between plasma CHIT and bone alterations is more controversial. Recent reports have shown that CHIT is produced by osteoclasts, and plays a role in their maturation and their resorptive activity. It has also been suggested that CHIT may serve as an useful serum marker for osteolysis (26). As mentioned, previous reports have shown higher osteoclastogenesis in PBMCs from GD patients (22,23). However, the clinical relevance of this observation has not been evaluated.

The aim of our work was to evaluate the correlation between pro-osteoclastogenic potential in GD patients with clinical bone parameters and CHIT activity levels. The results from this study would contribute to the understanding of bone pathophysiology in GD, looking for clinical implications of higher *in vitro* osteoclastogenesis.

2. Materials and methods

2.1 Patients and samples

A total of 29 GD1 patients (18 female and 11 male) were included in this study. All patients were under enzyme replacement therapy (ERT) with velaglucerase (VPRIV, Shire), although some of them had previously received imiglucerase (Cerezyme, Genzyme). Average time of ERT with velaglucerase was 3.5 years (range: 1-5). Patient demographic and clinical data are shown in Table 1. Splenomegaly and hepatomegaly were evaluated by quantitative abdominal MRI. Bone status is described according to bone densitometry and MRI studies. Twenty-five healthy volunteers were included as controls (Table 1). Healthy individuals were from Caucasian or South American origin (64% and 36%, respectively); while patients were from Caucasian (60%), South American (37%) or Ashkenazi Jewish origin (3%, corresponding to only one patient). No postmenopausal women were included in this study.

Peripheral blood samples from patients and healthy controls were obtained by venipuncture at morning after fasting overnight. Heparinized blood was used for PBMC isolation. Dried blood spot (DBS) on filter paper and serum were collected at the same time for CHIT activity determination and CTX, BAP or cytokine quantification, respectively. This study was approved by the Ethical Committee of IBYME (Instituto de Biología y Medicina Experimental, Argentina). All patients or their guardians provided written informed consent to participate in this study.

	Patients	Controls
Total included	29	25
Female (n, %)	18/29, 62%	15/25, 60%
Male (n, %)	11/29, 38%	10/25, 40%
Age (years): mean; range	23; 4-59	28; 9-59
Chitotriosidase		
Mean; range (µmol/l.h)	394; 48-2968	61; 24-110
Elevated levels (n, %)	23/29, 79%	0/25
Spleen and liver status (n, %)		
Splenectomized	2/29, 7%	
Non-splenectomized	27/29, 93%	
Splenomegaly	5/27, 18,5%	
Hepatomegaly	13/29, 45%	
Genotype (Allele frequency, %)		
R120W	2.3	
L444P	25	
N370S	38.6	
F411I	6.8	
R48W	2.3	
RecNcil	13.6	
T408M	2.3	
R121W	2.3	
R286C	2.3	
unknown	2.3	
Bone status		
BMD: Z-score < -1 (n, %)	5/18 (28%)	
Bone marrow infiltration (n, %)	6/18 (33%)	
BMB Score (total skeleton): mean; range	2; 0-8	
Patients with fragility fractures (n, %)	3/18 (17%)	
Patients with osteonecrosis (n, %)	4/18 (22%)	

Table 1. **Characteristics of patients and healthy individuals.** Data are shown as percentages and as numbers of patients over total patients tested for each parameter. BMD: bone mineral density; Z-score refers to total skeleton and/or lumbar spine. Bone marrow infiltration was assessed by MRI. Patients with fragility fractures included two patients with femoral fracture and one patient with vertebral fracture.

2.2 Osteoclast differentiation assay

PBMCs from patients and healthy controls were obtained by Ficoll Hypaque gradient separation (Sigma, St Louis, MO, USA). PBMCs were seeded at 500,000 cells per well in α-minimum essential medium (α-MEM) supplemented with 10% heat inactivated fetal bovine serum (Gibco-BRL, Life technologies, Grand Island, NY), 100 U/ml of penicillin and 100 µg/ml of

streptomycin and 30 ng/ml of recombinant human macrophage colony stimulating factor (M-CSF) (R&D, Minneapolis, MN, USA). Cultures were performed at 37°C in 5% CO_2 atmosphere for 14 days, replacing the media every 48 hs. The supernatant was harvested to assess CHIT activity. To evidence osteoclasts, cells were fixed in 4% paraformaldehyde and stained for tartrate-resistant acid phosphatase (TRAP; Sigma). Nuclei were stained with DAPI (Sigma). Samples were visualized in a Nikon Eclipse Ti fluorescence microscope with an X-Cites Series 120 Q light source. TRAP-positive multinucleated (more than three nuclei) cells were defined as osteoclasts. Counting was performed over a total of five 20x fields and results were normalized by total cellular area using image J software.

2.3 CHIT activity determination

CHIT activity was assessed in DBS and supernatant as follows: a 3 mm diameter from DBS filter paper or 20 µl supernatant was placed into a well of a black microplate and 40 µl of 0.25M sodium acetate buffer pH=5.5 and 40 µl of 0.19 mM 4-Metilumbeliferil β-D-N-N'-N''-triacetylchitotrioside (Sigma) were added. After an incubation for 30 min at 37°C, the stop solution (220 µl of 0.1 mol/l ethylenediamine, pH = 11.4) was added. The fluorescence of the product (excitation 365 nm; emission 450 nm) was measured on a Twinkle LB 970 fluorometer (Berthold Technologies, Bad Wild- bad, Germany). A standard curve of 4-methylumbelliferone (Sigma, Saint Louis, MO, USA) was used to extrapolate fluorescence counts to moles of enzymatic product. Enzymatic activity was expressed as micromoles of 4-methylumbelliferone produced per liter per hour.

2.4 Determination of serum cytokines, CTX and BAP

CTX and BAP were assessed in serum samples from healthy individuals and GD1 patients by electrochemiluminescence (Roche) and enzyme immunoassay (Abbot), respectively. Cytokines concentration in the serum samples were measured by ELISA. Assays for IL-6, TNF-α, transforming growth factor (TGF)-β, and IFN-γ were from BD Pharmingen, San Diego, CA; while IL-10 assay was from eBiosciences, San Diego, USA.

2.5 Bone Mineral Density and Magnetic Resonance Imaging

DXA was carried out on a Lunar Prodigy Advance (GE Healthcare) of total skeleton and lumbar

spine. To analyze the results in this study, patients were classified according to their Z-score values in lower or higher than -1. This criterion is based on the increased risk of fractures in GD1 patients with Z-score lower than -1 (27).

MRI of lumbar spine (sagittal), both femurs (coronal plane) and total skeleton were performed in an Achieva 1.5 Tesla instrument (Phillips Medical Systems). T1 and T2-weighted images obtained in spine and both femurs were analyzed to establish the BMB score described by Maas *et al.* and adapted by Robertson *et al*.(9,28) Up to eight points were assigned for each lumbar spine and femoral sites according to the degree of bone marrow infiltration (0/1 considered normal and 8 considered severe). Total BMB score was calculated as the BMB score from femur plus BMB score from lumbar spine.

2.6 Statistical analysis

Statistical analyses were performed with the Prism v.5.0 software (GraphPad software Inc., La Jolla, CA, USA). Two-tailed p values less than 0.05 were considered significant. Comparisons between osteoclasts number, CHIT activity, serum markers or cytokines from healthy controls and GD1 patients were performed using unpaired t tests. Correlation analyses were performed using Pearson's correlation test.

3. Results

3.1 Pro-osteoclastogenic potential and CHIT activity in GD1 patients

In agreement with previous reports (22,23), *in vitro* culture of patient´s PBMCs generated more osteoclasts than control cells (Figure 1A). As expected, CHIT activity in blood samples from patients was significantly higher compared to healthy controls (Figure 1B). We also evaluated the possible dependence of osteoclasts with age but no correlation was found ($r=0.0645$ and $p=0.7594$ for patients; $r=0.1692$ and $p=0.4187$ for controls). Similarly, blood CHIT activity did not correlate with age ($r=0.3561$ and $p=0.0535$ for patients; $r=0.3722$ and $p=0.1167$ for controls).

As recent reports have shown a physiologic role of CHIT in osteoclast maturation (26), we evaluated CHIT activity in the supernatants from mature osteoclasts generated from patient and control´s PBMCs. Surprisingly, CHIT activity presented no difference between patients and controls (Figure 2A). Despite this observation, a correlation between osteoclast´s number and CHIT activity in the supernatant was observed in controls, while GD1 patients showed a similar trend (Figure 2B). On the other hand, we hypothesized that osteoclasts may contribute to blood CHIT activity, but no correlation was observed between supernatant and blood´s CHIT activities ($r=0.0980$ and $p=0.7084$ for controls, $r=0.2100$ and $p=0.3742$ for patients). In addition, no relation was found between osteoclast´s number and blood CHIT activity (Figure 3).

3.2 Bone mineral density correlates with pro-osteoclastogenic potential but not with blood CHIT activity

We evaluated the relation between pro-osteoclastogenic potential (expressed as osteoclast number) and BMD, expressed as Z-score. Remarkably, correlation analysis between these two parameters from patients showed a significant inverse correlation: both total skeleton and lumbar spine Z-scores decrease as osteoclasts increase (Figure 4A). Moreover, patients with Z-score (total skeleton or lumbar spine) lower than -1 presented higher numbers of osteoclasts than those with Z-score higher than -1 (Figure 4B). This is of particular interest since Z-scores lower than -1 have been associated with an increased risk of fractures in GD1 patients (27). MRI can also be used for monitoring bone status in GD1 (10,29), then we also evaluated the pro-osteoclastogenic potential in patients with an altered BMB score from total skeleton or normal BMB score, but no difference was observed (Figure 4C). Indeed, no correlation was found between BMB score and osteoclasts ($r=0.2819$ and $p=0.2901$; not shown). Finally, we

evaluated blood CHIT activity levels in relation to Z-score. However, no correlation was found (Figure 4D).

3.3 Increased osteoclastogenic potential involves higher levels of bone remodeling markers

We assessed CTX and BAP levels in serum samples from GD1 patients and healthy controls. Both groups showed a negative correlation with age for both markers (CTX: r=0.8100 and p=0.0001 for patients, r=0.5595 and p=0.0375 for controls; BAP: r=0.6000 and p=0.0085 for patients, r=0.5670 and p=0.0334 for controls). Due to the age-dependence, we compared the biomarkers levels in control and GD1 individuals younger (pediatric) or older (adults) than 20 years old. Adult group included 14 GD1 patients (mean age: 30 years old, range 21-54) and 10 healthy individuals (mean age: 35 years old, range 22-59); while pediatric group included 10 GD1 patients (mean age: 9 years old, range 5-14) and 4 healthy individuals (mean age: 13 years old, range 9-16). We found that CTX and BAP levels were significantly higher in GD1 adult patients than in controls (Figure 5A and 5B). CTX and BAP levels in the pediatric group showed a similar trend (Figure 5A and 5B); however, these results should be interpreted with caution due to the small number of pediatric controls included in this study. In addition, we also observed a correlation between osteoclast numbers and serum CTX in the GD1 pediatric group (Figure 5C) but not in adult patients or controls (not shown). Serum BAP levels behaved similarly to CTX, although not statistical difference was found (Figure 5C).

3.4 Serum cytokine profile in patients reveals a pro-osteoclastogenic status and a correlation with BMD

Considering the involvement of immune molecules in bone remodeling, we decided to evaluate the concentration of TNFα, IL-6, TGFβ, IL-10 and IFNγ in serum samples (Figure 6). Compared to controls, GD1 patients presented higher levels of IL-6, and lower levels of TGFβ and IL-10. Differences in TNFα and IFNγ levels were not statistically significant. Remarkably, IL-10 and IFNγ -negative regulators of osteoclastogenesis- positively correlated with Z-scores values from total skeleton (Figure 7A): as Z-score increases, IL-10 levels also increase; and the same situation was observed for IFNγ levels. In addition, we compared cytokine levels between patients with Z-score lower than -1 and patients with Z-score higher than -1 (Figure 7B). As expected, the first group of patients presented a trend towards decreased levels of IL-10 and IFNγ, but also IL-6. Furthermore, IL-6 serum levels negatively correlated with the number of osteoclast generated *in vitro* (p=0.0125; r=-0.5019).

4. Discussion

Pathophysiology of skeletal alterations in GD1 is still poorly understood. Previous studies have evaluated osteoclastogenesis in different *in vitro* experiments and revealed that GD1 PBMCs secrete pro-osteolastogenic factors (30). Moreover, GD1 patients display higher numbers of circulating osteoclast precursors with a higher tendency to differentiate into functional osteoclasts (22,23). Taken together, these reports show a misbalance of bone homeostasis through higher resorption. We performed this study to evaluate the relation between the increased osteoclastogenic potential and clinical bone parameters in GD1 patients. We especially focused on BMD, a factor affected early during GD1 bone alterations. Our results showed for the first time that the increased differentiation of osteoclast precursors from GD1 PBMCs correlates with poor BMD, as evaluated by Z-score from both, total skeleton and lumbar spine. The International Collaborative Gaucher Group (ICGG) Gaucher Registry revealed that osteopenia develops in childhood (5). ICGG also found that signature skeletal complications of GD1 were unrelated to standard measures of disease severity, such as visceral involvement, serum CHIT, or even genotype. The only risk factor for fractures was lumbar spine BMD (27). This study reinforces the idea that BMD is a main aspect of skeletal complications. We also studied the relation of osteoclast numbers with MRI studies, but our work showed no correlation between MRI evaluation and proosteoclastogenic potential. This observation is in agreement with Mariani *et al.* who established that the results of bone marrow imaging could not be directly correlated to a reference standard for the actual burden of Gaucher cells (31). However, previous work by Reed *et al*. showed that pro-osteoclastogenic potential was higher in patients with MRI-evidence of active bone disease than in those patients without it (23). Probably this discrepancy is because our study design involved not only the number of osteoclast precursors but also the microenvironment created by other PBMCs.

The usefulness of bone turnover markers in GD1 is still unclear. Markers of bone formation in treatment-naive patients are usually normal or decreased, whereas markers of bone degradation are normal or increased (1). Therefore, GD1 patients would present both, increased bone degradation and impaired bone formation, leading to osteoporosis (32). Because previous studies are not conclusive, we evaluated serum markers in our cohort of patients. Our results showed that CTX is increased in adults with GD1 and suggest the same for children. In addition, a positive correlation between CTX and the number of osteoclasts generated *in vitro* was observed in children. On the other side, BAP showed higher levels in adult patients than in controls. These results reinforce the idea that higher remodeling is at least one of the hallmarks leading to osteopenia/osteoporosis in GD1.

CHIT is produced and secreted by lipid laden macrophages or Gaucher cells and the determination of its activity in blood reflects total body burden of Gaucher cells. There is relative discrepancy about the usefulness of CHIT as biomarker of skeletal problems. For example, Roca *et al*. reported the absence of correlation between CHIT and bone marrow fat fraction (33), and van Breemen *et al.* showed no efficiency of CHIT in the follow up of skeletal disease (34). On the contrary, Pavlova *et al.* showed a correlation between CHIT and osteonecrosis (35); while van Dussen *et al.* reported significantly reduced CHIT values in patients with no bone complications as compared to patients with bone problems at baseline or during ERT (25); however significant overlap was found between these two groups and bone alterations were studied as a whole group, without distinction among clinical parameters. In addition, CHIT was shown to be functionally involved in the osteolytic process and correlates with resorptive activity of the osteoclasts (26). Due to osteoclasts are derived from the macrophage lineage and CHIT plays a role in normal osteoclastogenesis, we hypothesized that osteoclasts could be a main source of CHIT, either in blood or extracellular space, and consequently blood CHIT could correlate with bone problems. Our results completely abolish this idea. Although CHIT activity in blood is higher in GD1 as compared to controls, no correlation was observed between blood CHIT and the number of osteoclasts produced *in vitro*. Moreover, CHIT activity in the supernatant from osteoclasts differentiation assays increases as more osteoclasts are produced, irrespective of the source of PBMCs. Consequently, neither blood CHIT nor supernatant CHIT reflected the increased number of osteoclasts obtained from GD1 cells.

Finally, as bone remodeling and immune system are closely related, we studied the cytokine profile in GD1 patients. Increased osteoclastogenic potential accompanied by altered cytokine profile was reported for other pathologies (12). Proinflammatory status in GD1 was confirmed by different studies, although there is no consensus on what cytokines -if any- are the main mediators of bone damage (21,35,36). In this study, we evaluated the pro-osteoclastogenic cytokine TNFα but surprisingly its levels were not different between GD1 patients and controls. On the contrary, we confirmed elevated levels of serum IL-6 in GD1 patients. IL-6 is known to enhance RANKL secretion from osteoblast and stromal cells but at the same time it can negatively act over osteoclast precursors (16). In fact, despite observing increased levels of IL-6 in serum from GD1 patients, we observed a negative correlation between this cytokine and osteoclastogenic potential. These results highlight the pleiotropic role of IL-6 in bone remodeling and suggest a predominant protective role for IL-6 in GD1, particularly evidenced by those patients with higher Z-scores presenting higher levels of IL-6. Similar results were report for different pathological conditions with bone compromise such as rheumatoid

arthritis (37). IL-10, TGFβ and IFN-γ act as early inhibitors of osteoclast differentiation (14,15,38,39), and presented lower levels in patients compared to controls, suggesting that an impaired negative regulation of osteoclastogenesis could contribute to the increased osteoclastogenic potential observed in GD1 patients. Moreover, IL-10 and IFN-γ presented an inverse correlation with BMD expressed as total skeleton Z-score.

This study presents some limitations given by the characteristics of the group of patients: patients of both genders in a wide range of age were included. In addition, ERT time heterogeneity of patients could affect bone status in different ways. Nevertheless, by analyzing the whole group we can observe that an altered BMD is accompanied by an altered osteoclast generation. Further studies with a wider group of subjects, including more patients with poor BMD, would help to clarify the effect of ERT time on osteoclast potential.

In conclusion, our study shows for the first time that the increased differentiation of osteoclast precursors from peripheral blood from GD1 patients correlates with poor BMD, evaluated as Z-score both total skeleton and lumbar spine. On the contrary, CHIT activity does not correlate with BMD. In addition, an altered immune regulation in GD1 may play a significant role in bone pathophysiology although further studies are needed regarding the osteoimmunology of GD1 in order to elucidate the pathological mechanism and find new complementary therapies.

Author Contributions

PR, DG and BO designed the protocol of the study, invited physicians and recruited patients, reviewed and analyzed the results, and wrote and revised the article.

CB and JM performed lab experiments, reviewed and analyzed the results, and wrote and revised the article.

AC, MO and RC performed lab experiments and reviewed and analyzed the results.

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Declaration of interests

PR, BO and DG had received grants from Shire. CB, JM, AC, and RC declare no conflict of interest.

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Figures´ legends:

Figure 1. Osteoclasts generated from PBMCs in vitro and blood CHIT activity. A) Osteoclast´s counting after 14 days-culture of PBMCs from healthy controls (n=25) or GD1 patients (n=29) in the presence of M-CSF. **B)** CHIT activity from blood samples was determined. Unpaired t test; ***p<0.001; **p<0.01.

Figure 2. CHIT activity in supernatant and its correlation with osteoclast number. A) CHIT activity was evaluated in mature osteoclasts´ supernatant from controls (n=25) and patients (n=29). **B)** Correlation between osteoclasts and secreted CHIT activity for controls and GD1 patients. Pearson´s coefficients and p values are shown.

Figure 3. Correlation between blood CHIT activity and *in vitro* generated osteoclasts. Correlation between CHIT activity from blood samples and the number of osteoclasts generated *in vitro* from PBMCs. White circles (controls, n=25); black circles (GD1 patients, n=29). Pearson´s coefficients and p values are shown.

Figure 4. Osteoclasts correlate with bone mineral density but not with blood CHIT activity. A) Correlation between osteoclasts and bone mineral density expressed as Z-score from total skeleton or lumbar spine in GD1 patients (n=18). Pearson´s coefficients and p values are shown. **B)** Osteoclasts from patients grouped according to Z-score values. Black bars: total skeleton Z-score lower (n=3) or higher (n=15) than -1. Grey bars: lumbar spine lower (n=4) or higher (n=14) than -1. Unpaired t test; *p<0.05. **C)** Patients were grouped according to the presence (n=6) or absence (n=12) of an altered BMB score in MRI studies and osteoclasts numbers were depicted, non-statistical difference was observed (unpaired t test, p=0.2384). **D)** Correlation between Z-score values from total skeleton or lumbar spine and blood CHIT activity in GD1 patients (n=18). Pearson´s coefficients and p values are shown.

Figure 5. Biomarkers of bone resorption and formation are altered in GD1 patients. Serum CTX **(A)** and BAP **(B)** levels were evaluated in patients and controls. Data were evaluated in two age groups: younger (pediatric) and older (adults) than 20 years old. **C)** Correlation between osteoclasts and CTX or BAP levels in pediatric GD1 patients. Pearson´s coefficients and p values are shown.

Figure 6. Cytokines involved in bone metabolism are altered in GD1 patients. Cytokine levels were assessed by ELISA in serum samples from patients (black bars, n=29) and controls (white bars, n=25). Unpaired t test, *p<0.05; **p<0.01.

Figure 7. Relation between cytokines and BMD. A) Correlation between IL-10 or IFNγ and BMD expressed as Z-score from total skeleton. Pearson´s coefficients and p values are shown. **B)** Serum cytokines levels in patients with Z-score lower than -1 and patients with Z-score higher than -1. Unpaired t test, differences were not statistically significant.

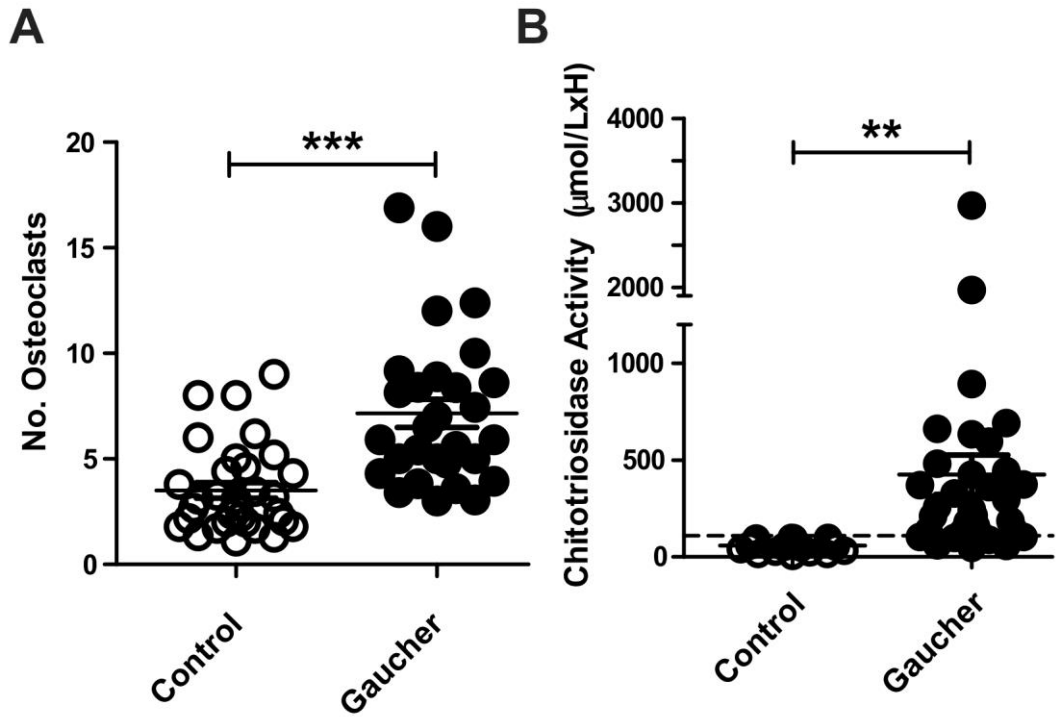

Fig. 1

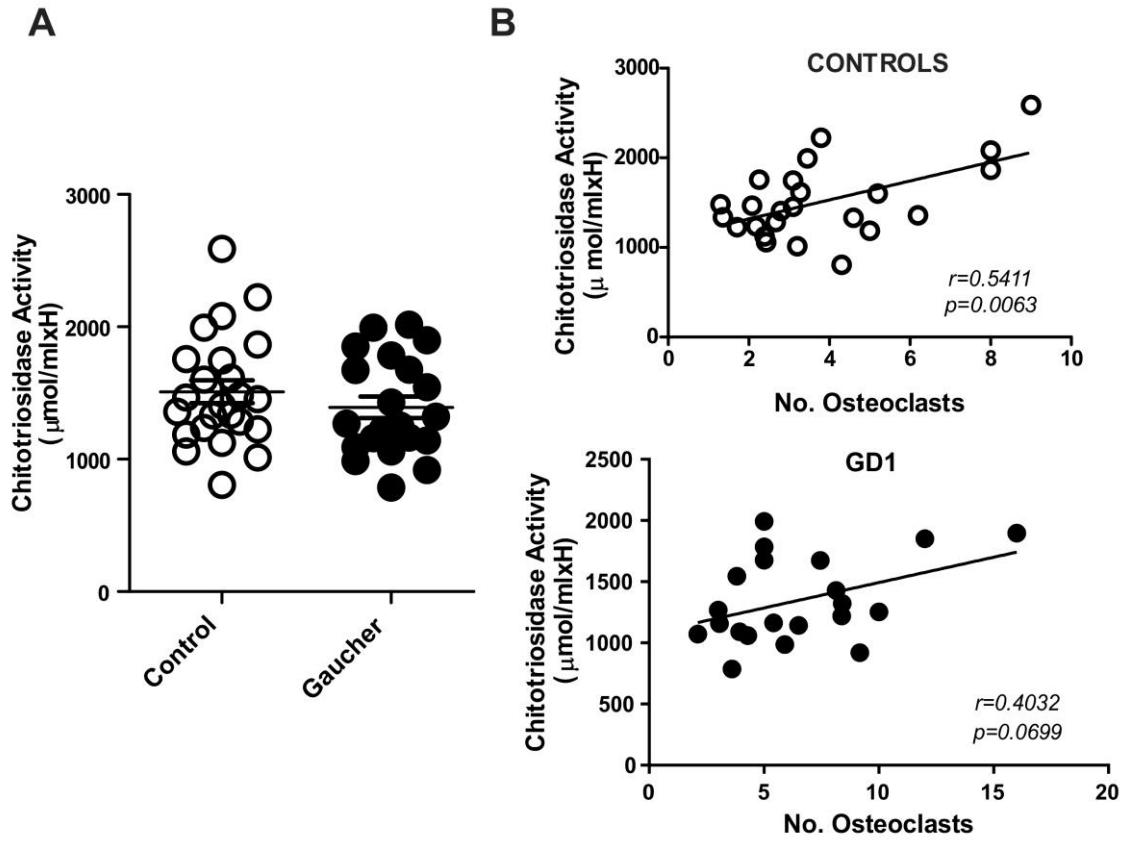

Fig. 2

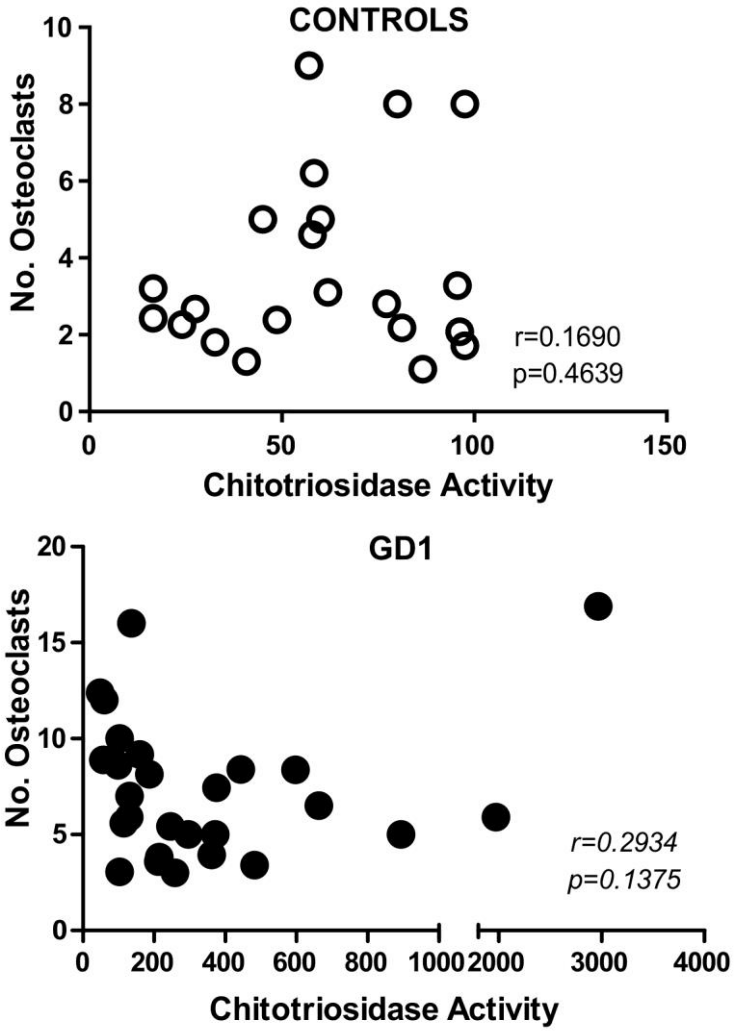

Fig. 3

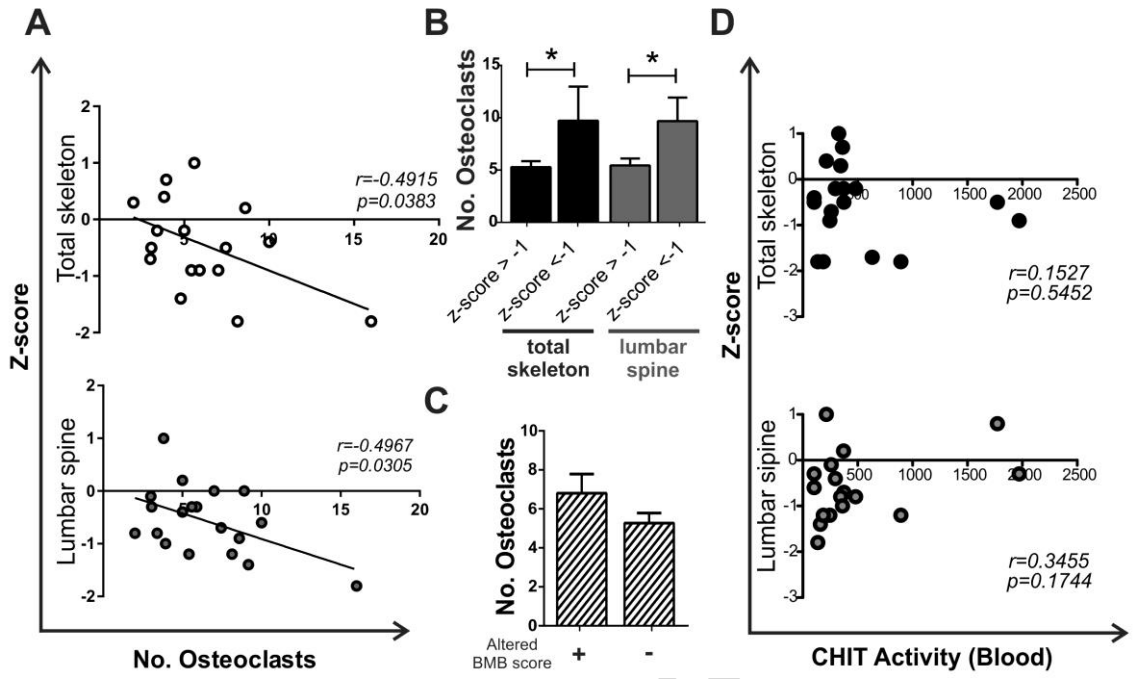

Fig. 4

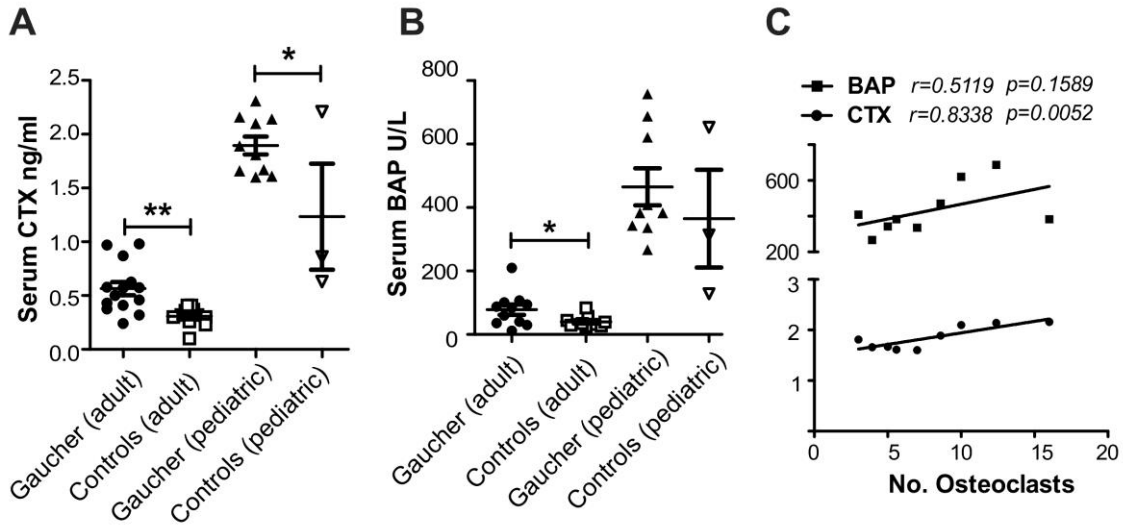

Fig. 5

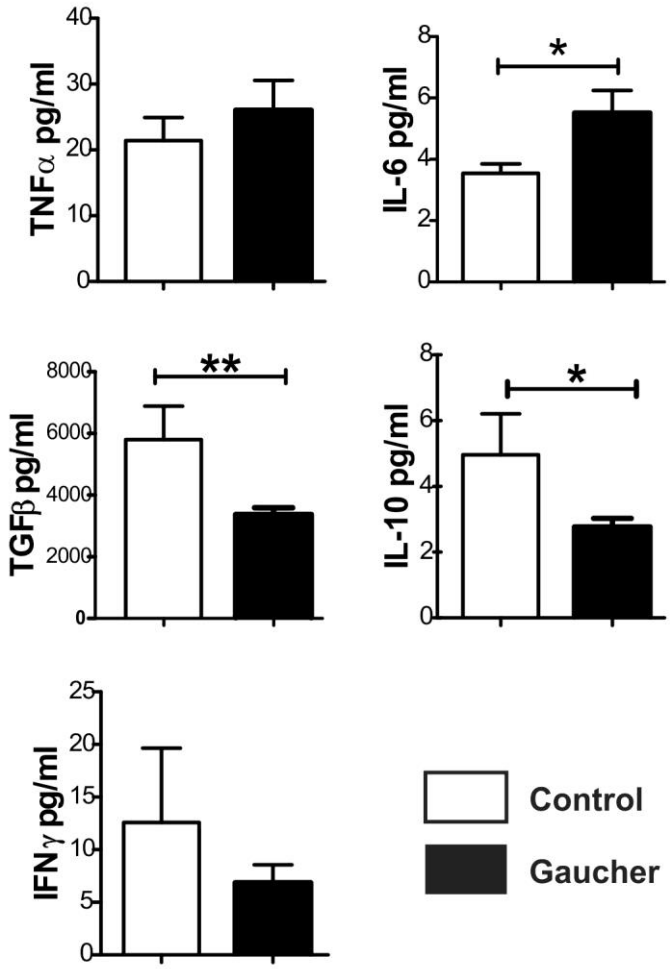

Fig. 6

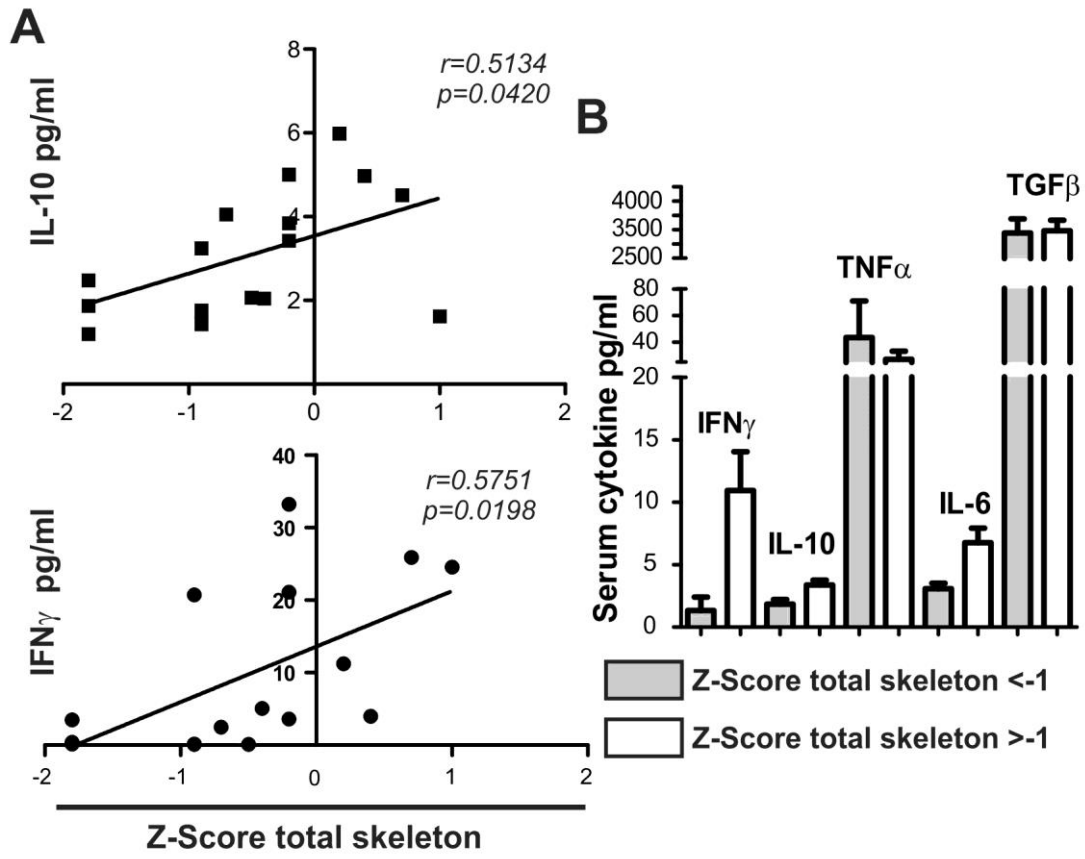

Fig. 7

Highlights

- Increased osteoclastogenic potential from patients with GD1 associates with poor BMD.
- CHIT values are not correlated with BMD or pro-osteoclastogenic potential.
- Serum cytokines are altered in GD and could play a key role in bone pathology of GD.